Morphological Changes in Rat Hippocampus after Brain Injury

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We performed morphological analysis of the structure of rat hippocampus after ablation of the left sensorimotor cortex. Four experimental groups were formed: two control groups (intravenous and intracerebral injections of the culture medium) and two experimental groups (intravenous and intracerebral transplantation of MSC). Ten weeks after surgery, disturbed cytoarchitectonics and great number of dead neurons were found in all zones of the hippocampus in animals of the control groups. In animals receiving cell therapy, no pathological changes in the structure of the hippocampus were found: hyperchromatic neurons were absent and the cells had regular shape and closely adjoined to each other.

Key Words: *mesenchymal stem cells; sensorimotor cortex ablation; hippocampus*

In 1970s, special cells, so-called place cells, were detected in rat hippocampus; electrical activity of these cells increased in response to novel environment [9]. It was hypothesized that the main function of the hippocampus is processing and storing of the information about the environment, orientation, navigation, *etc*. It was hypothesized that hippocampus may act as a cognitive map of the environment. Similar cells were later found in human hippocampus [6]. Place cells are diffusely spread in all zone of the hippocampus: they are presented by pyramid cells in CA1 and CA3 and by granular cells in the dentate gyrus. Damage to the hippocampus has a negative impact on cognitive and orientation-exploratory behavior in animals and humans and impairs short-term behavior, learning, and spatial and temporal orientation [5]. Experiments on various models of traumatic brain injury show that morphological changes in the hippocampus appear not only after direct mechanical damage to this structure or adjacent brain areas, but also in cases when the focus of injury is located far from the hippocampus [1].

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Here we studied morphological changes in rat hippocampus developing over 10 weeks after sensorimotor cortex ablation (SCA) in the left hemisphere and the effects of intravenous and intracerebral transplantation of MSC on the state of the hippocampus.

MATERIALS AND METHODS

Experiments were carried out on 40 male Wistar–Kyoto rats weighing 200-250 g. The animals were maintained under standard vivarium conditions with free access to food and water. The study was performed in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986). The study protocol was approved by ethical committee.

The sensorimotor cortex of the heft hemisphere was removed as follows. The rats were intraperitoneally anesthetized with 30 μl Zoletil (Zoletil 100, Virbac Sante Animale). A hole (2×3 mm) was drilled in the left parietal bone, the dura matter and the entire motor cortex were removed (AP=(-1)-(-4) mm from bregma; SD=1.0 mm laterally from the sagittal cranial suture). The depth of ablation did not exceed 2 mm. The skin was sutured.

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MSC were routinely isolated from rat BM [2] and immunophenotyped by the method of flow cytofluorometry on an Epics XL flow cytofuorometer (Becton Coulter). MSC culture consisted of $CD90^+$ cells (97%, of them 15% cells were $CD106^+$) and $CD45^+$ cells (3%, hemopoietic cells).

The animals were divided into 4 groups. Group 1 animals (control I; n=10) received 100 μl culture medium into the caudal vein after SCA; group 2 animals (cell therapy I; n=10) after SCA received 5×10^6 MSC in 100 μl culture medium; group 3 animals (control II; n=10) received intracerebral injection of culture medium (20 μl); and group animals (cell II; n=10) received intracerebral injection of MSC suspension (200,000 MSC in 20 μl culture medium).

Intracerebral transplantation consisted of two shots (10 μl culture medium or cell suspension each) into brain areas adjacent to the damaged area and located frontally and dorsally to it at a depth <2 mm (*i.e.* strictly within the neocortex).

The animals were euthanized 2 and 10 weeks after SCA by decapitation, the brain was removed, and the fragment including the damaged area and adjacent zones was isolated. The brain was fixed in Zn-formalin for 1 day, dehydrated in ascending alcohols, embedded in paraffin blocks, and 5-7-μ sections were prepared and stained with toluidine blue after Nissl.

Analysis of hippocampal structures was performed at a level of -1.14 mm from bregma.

RESULTS

The procedure of SCA in the left hemisphere was carried out so that the damage was localized in the neurocortex and did not involve the outer capsule. Neither SCA, nor MSC transplantation caused mechanical damage to the hippocampus.

Morphological analysis of brain sections from group 1 animals (control I) 2 weeks after SCA revealed great number of hyperchromatic neurons in CA1 and dentate gyrus. Layer cytoarchitectonics was disturbed, pronounced edema was observed in the dentate gyrus (Fig. 1, *a*). In group 2 (cell therapy I), no hyperchromatic cells were observed in CA1 and dentate gyrus, the neurons had regular shape and closely contacted to each other (Fig. 1, *b*). In group 3 (control II), abundant hyperchromatic neurons were seen in CA1. Cytoarchitectonics was markedly altered (neuron-free areas were observed). Solitary hyperchromatic cells were found in the dentate gyrus. No pronounced edema was observed (Fig. 1, *c*). In group 4 (control II), only few hyperchromatic neurons were seen in CA1, but the distance between neurons was larger. No morphological changes in dentate gyrus were detected (Fig. 1, *d*).

In group 1 (control I), structural abnormalities were observed in all zones of the hippocampus 10 weeks after SCA, cell-free fields were found. In CA1 and dentate gyrus, numerous dying hyperchromatic neurons were seen (Fig. 2, *a*). Less pronounced morphological changes in the structure of the hippocampus were observed in group 2 (cell therapy I): only solitary dead neurons were detected in CA1 and dentate gyrus, cytoarchitectonics of the hippocampal zones was only little changed (Fig. 2, *b*). In group 3 (control II), abundant hyperchromatic neurons were seen in both CA1 and dentate gyrus. Cytoarchitectonics of hippocampal layers was disturbed (Fig. 2, *c*). At the same time, no pathological changes in the hippocampal structure were found in group 4 animals (cell therapy II): hyperchromatic neurons were absent and the cells had regular shape and closely adjoined to each other (Fig. 2, *d*).

Thus, our study showed that SCA had a negative impact on morphological state of all hippocampal zones: progressive neuronal death in CA1 and dentate gyrus was observed during 10 weeks after the intervention. After intravenous or intracerebral transplantation of MSC, neuronal death was less pronounced during the first weeks after the intervention, while by the 10th week practically all hippocampal cells had normal morphology and formed dense layers. Hence, MSC transplantation had a protective effect not only on neurons located in immediate proximity to the area of damage, as it was previously demonstrated on the models of ischemic stroke and minor trauma to the sensorimotor cortex [10], but also on nerve cells in distant brain structures.

Some trophic factors, in particular, GDNF, BDNF, NGF, EGF, and bFGF produce a protective effect due to binding of free radicals, inhibition of apoptosis, and acceleration of the course of the inflammatory reaction [7]. MSC secrete these substances *in vitro* and probably can synthesize them in the site of injury, thus inhibiting apoptosis and improving viability of damaged cells [4].

Moreover, the hippocampus is a structure where neurogenesis is going on in adult animals [8]. We have previously demonstrated that transplantation of MSC activated neurogenesis in the subventricular zone of lateral ventricles after ischemic stroke [10]. It can be hypothesized that MSC produce a stimulatory effect on the hippocampus and neuronal loss in the dentate gyrus caused by SCA can be to certain extent replenished due to newly formed cells.

Structural integrity of the hippocampus after traumatic brain injury is crucially important for the maintenance of cognitive and orientation-exploratory behavior at the level observed in intact animals. Openfield testing showed that the number of the main behavioral acts decreased by 2-4 times after SCA of the left hemisphere. The animals were inactive, did not

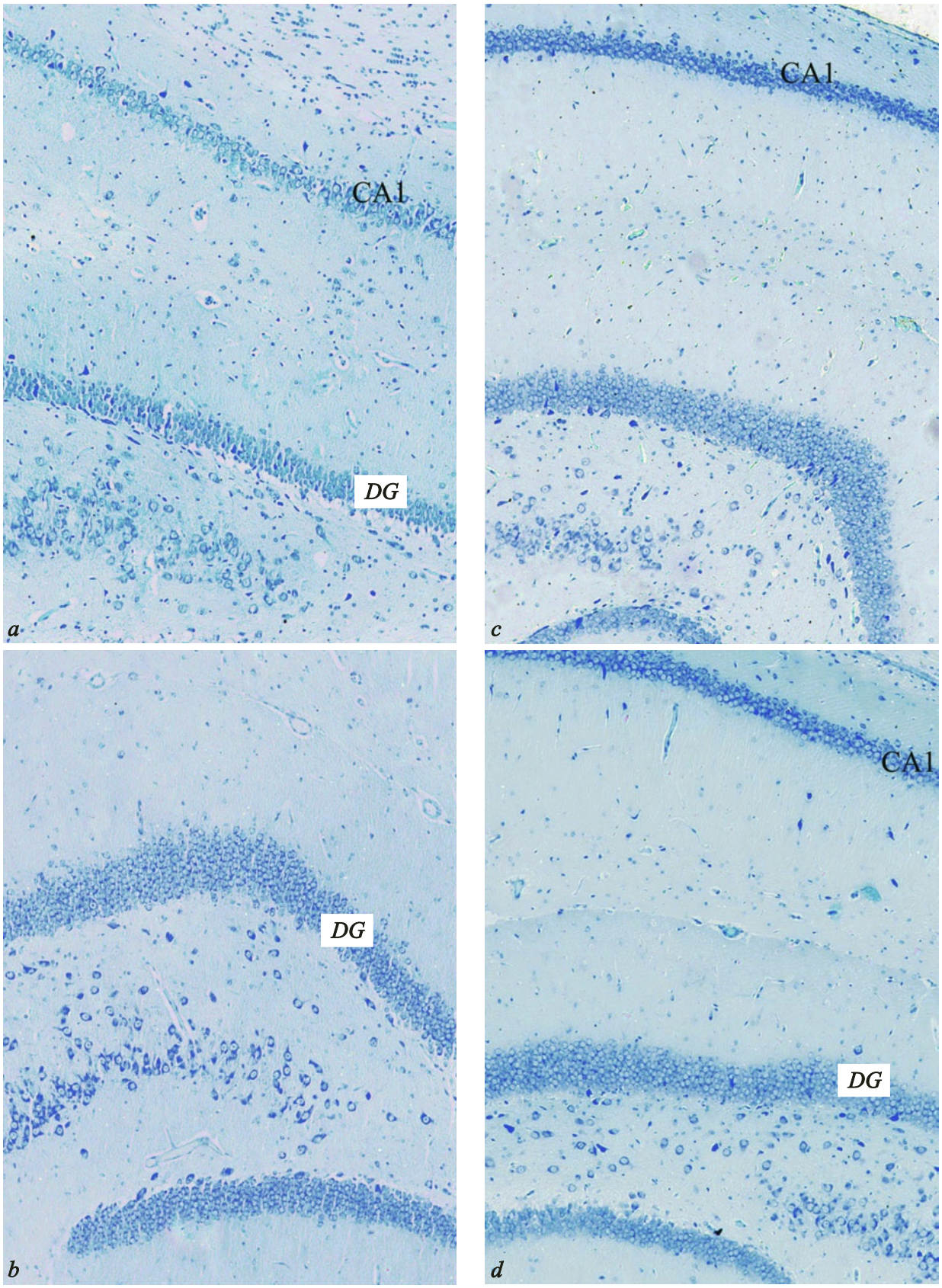

Fig. 1. Rat hippocampus 2 weeks after SCA. Here and in Fig. 2: *a*) group 1 (control I); *b*) group 2 (cell therapy I); *c*) group 3 (control II), *d*) group 4 (cell therapy II). Toluidine blue staining after Nissl; objective ×10. *DG*: dentate gyrus.

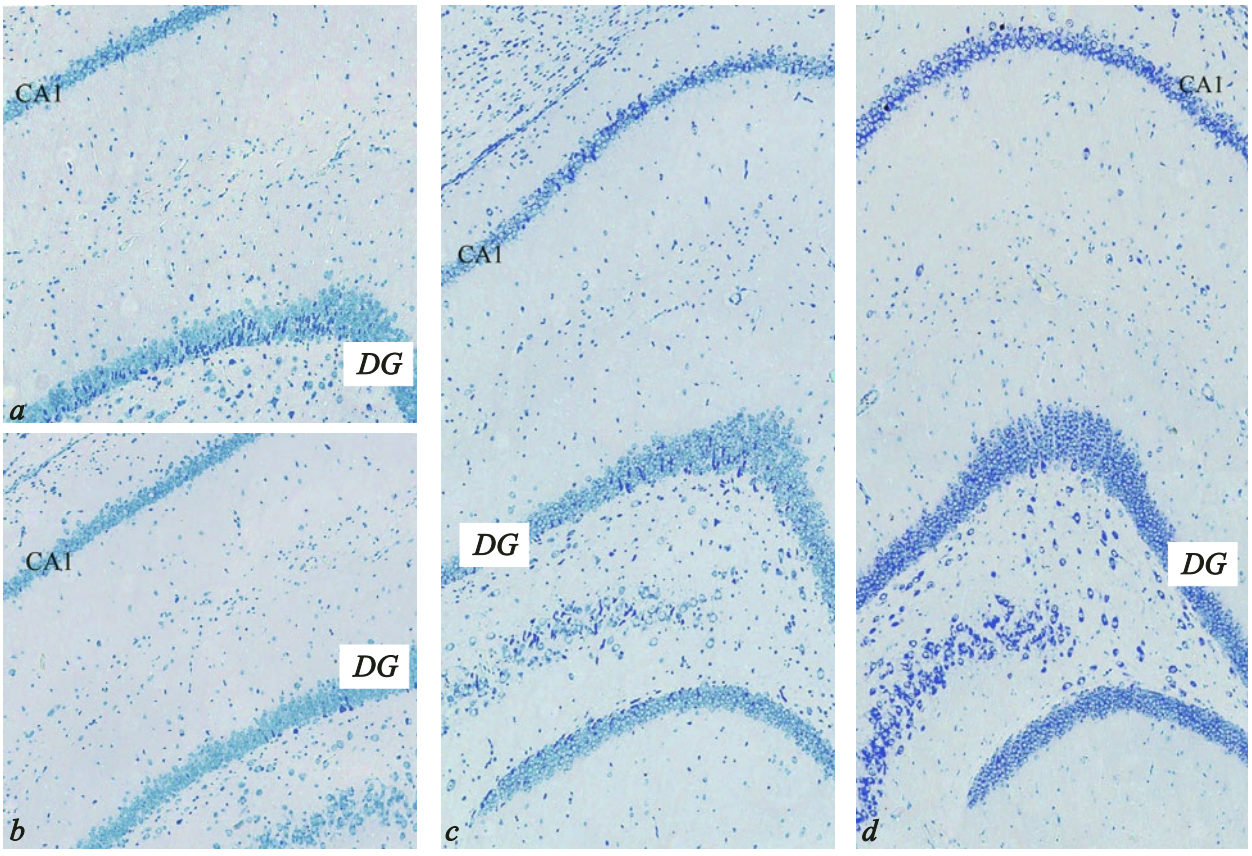

Fig. 2. Rat hippocampus 10 weeks after SCA.

explore the field, spent long time before they started to move, and most of the time remained motionless. After MSC transplantation into the caudal vein, motor and exploratory activities of the experimental animals decreased by 1.5-2 times during the first 2 weeks, but then remained at this level to the end of the experiment. Intracerebral MSC transplantation prevented behavioral disturbances starting from the first weeks after SCA and after 10 weeks the number of behavioral acts in these rats did not differ from that in intact animals.

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